Genome Biology

Meeting report

Defining and targeting transcription factors in cancer

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Abstract

A report from the Keystone Symposium on Molecular and Cellular Biology, 'Deregulation of transcription in cancer: controlling cell fate decisions', Killarney, Ireland, 21-26 July 2009.

This Keystone meeting focused on mechanisms of transcriptional regulation, the effects of transcriptional deregulation and the consequences on cancer, and the possibilities for inhibiting transcription factors as potential therapeutic targets. Greg Verdine (Harvard University, Cambridge, USA) suggested that, until now, only 20% of the genome was targetable with drugs (druggable). Transcription factors have traditionally been considered too difficult to target, but with increased understanding of transcription factor biology and technological advances, targeting them is becoming a realistic option.

The role of polycomb complexes in regulating transcriptional activity was an important theme to the meeting. Kristian Helin (University of Copenhagen, Denmark) summarized the role of polycomb repressor complexes (involving EZH2) in controlling senescence, in particular their role in the regulation of the $p16^{INK4A}$ and $p14^{ARF}$ tumor suppressor proteins. Competitive binding between polycomb repressor complexes and the histone demethylase JMJD3 determines transcriptional activity, suggesting that JMJD3 can act as a tumor suppressor. Maarten Van Lohuizen (Netherlands Cancer Institute, Amsterdam, The Netherlands) presented work on BMI1, a member of the polycomb repressor complex PRC1, which is crucial in the maintenance of adult stem cells. Loss of BMI1 in mouse models causes a dramatic reduction in proliferation in the mammary gland. Levels of BMI1 and EZH2 and interactions between them influence tumor progression. Qiang Yu (Genome Institute of Singapore, Singapore) focused on the possibility of targeting EZH2 in cancer. Their compound, 3-deazaneplanocin A (DZNep), an *S*-adenosylhomocysteine hydrolase inhibitor, depletes EZH2 in breast cancer cells. In colorectal cancer, DZNep reactivates the tumor suppressor microRNA *miR449*, leading to cell cycle arrest. He suggested that DZNep, in combination with 5-azacytidine, may be an effective cancer treatment.

Several talks used contemporary genomics technologies to define transcription factor binding sites, regulatory regions and changes in chromatin structure, all of which can contribute to altered cellular growth. Manel Esteller (Catalan Institute of Oncology, Barcelona, Spain) has assessed global DNA methylation in normal and cancer cells and found significant changes in DNA methylation at promoters. He discussed the DNA epigenome project, an ambitious project that will study the methylation state of 10,000 promoters in tumors from more than 1,000 patients. Bing Ren (University of California, San Diego, USA) discussed global DNA methylation and histone modification data generated by chromatin immunoprecipitation microarrays (ChIP-chip) or ChIP-sequencing (ChIP-seq). In human embryonic stem cells that were induced to differentiate, he showed that 30,000 putative enhancers exist in pre- and post-differentiation states, but only 8,000 of these were common between the two states. Within the regions shown to have altered chromatin structure following differentiation, he could show enrichments of motifs for various transcription factors. Both positive and inverse correlations were found when the histone maps were combined with DNA methylation data. Importantly, he could show that in specific cell types, non-CG methylation could occur and this was usually depleted from promoters of actively transcribed genes.

Two talks from members of the Genome Institute of Singapore highlighted new data on estrogen receptor (ER) transcription and chromatin dynamics. Yijun Ruan presented data on a novel technique called 'whole genome chromatin interaction analysis using paired-end ditagging' (ChIA-PET), which is a global method for identifying chromatin loops that form during transcription. By applying this to estrogen-induced gene transcription in breast cancer cells, his group found many hundreds of estrogen-induced intrachromosomal chromatin loops that form over distances as great at 1 Mb, representing *cis*-regulatory components that physically interact. As a follow-up to this presentation, Ed Liu presented data from recent genome-wide mapping of ER binding sites. He showed that only small subsets of predicted motifs are actual binding sites *in vivo* and that the ER binding sites

occur in gene-rich areas. Within the list of ER binding sites, the strongest (that is, those most enriched by ChIP-seq) were more likely to contain responsive motifs and to be adjacent to genes that are differentially regulated by estrogen. This suggests that there is a hierarchy of ER binding sites, in which the strongest sites are more likely to be functional, possibly as a result of superior ER-DNA interactions.

Maintaining the theme of nuclear receptor transcriptional activity, Ralf Kittler (University of Chicago, USA) has engineered bacterial artificial chromosomes of more than 20 different nuclear receptor (NR) genes to include an enhanced green fluorescent protein tag, and these have subsequently been used for genome-wide mapping using ChIP-chip. By correlating the binding profiles of all NRs, they found profile similarities and common binding sites between ER and retinoic acid receptor α; they hypothesized that this was an antagonistic interaction.

Arul Chinnaiyan (University of Michigan, Ann Arbor, USA) presented data characterizing gene fusions in prostate cancer, including his group's original discovery of fusions between the *TMPRSS2* gene (which encodes a transmembrane serine protease) and the *ERG* ETS-family oncogene in prostate cancers. He showed the results from large-scale genomic screens, which have resulted in more than 100 validated gene fusions. Interestingly, all of the fusions contain an ETS factor as the transcriptionally active partner and the other half of the fusion is usually an androgen-regulated gene target. He showed that fusions of various kinds are found in more than half of all prostate cancers and that their specificity to cancer and not normal tissue has led to a urine-based test for detecting fusion genes that may function as a prognostic indicator of prostate cancer. One of the major issues that researchers in the field face is defining which fusions are driving factors and which ones are passenger events.

One of the key themes of the meeting was the ability to target transcription factors using drugs. Traditionally, transcription factors were generally considered too difficult to target, and kinase pathways or cell surface proteins have instead been popular therapeutic targets. However, transcription factors are the downstream effectors of many pathways and this, coupled with technological advances, has made them attractive and realistic drug targets. Greg Verdine presented data on 'stapled peptides'. Normally, peptides used to block or inhibit transcription factors are easily degraded or have poor solubility. Verdine's approach provides a stabilizing backbone (or 'staple') to the short peptides or proteins, thereby generating proteins that are stable and maintain correct conformation. His group has successfully developed stapled peptides that target Bcl-2, Bax and BID (proapoptotic proteins containing only the BH-3 motif). One of their inhibitors is about to enter clinical trials, and the approach provides a realistic option for targeting a specific transcription factor in cancer.

John Rossi (Beckman Research Institute of the City of Hope, Duarte, USA) discussed his group's RNA interference method as a potential therapeutic approach. He suggested that the delivery of small interfering RNAs (siRNAs) to target cells has improved considerably, but one of the major problems is producing sufficient quantities of any specific siRNA. He also showed that they can generate siRNAs that target two different transcripts simultaneously, allowing increased effectiveness from a single siRNA. Lyubomir Vassilev (Roche Pharmaceuticals, Nutley, USA) showed data on Nutlin, an inhibitor of the interaction between the p53 tumor suppressor and its regulator Mdm2. This inhibitor binds selectively to the pocket of Mdm2, resulting in increased p53 levels. Nutlin was an effective inhibitor in cell lines and in mice with various types of cancer and was bioavailable when administered orally. These data confirm that protein-protein interactions between transcription factors and regulatory proteins can be blocked successfully with chemical inhibitors. Sandra Dunn (University of British Columbia, Vancouver, Canada) provided compelling evidence for an important role for the transcription factor YB-1, which is expressed in 40% of breast cancers but not normal breast tissue. YB-1 was shown to correlate with poor prognosis in patients and in mouse models, and YB-1 transgenic mice readily generated tumors. YB-1 activity is dependent on phosphorylation, and Dunn's group has shown that the ribosomal S6 kinase (Rsk) complex is involved. They are currently testing the effectiveness of peptides that target YB-1.

René Bernards (Netherlands Cancer Institute) stressed the need to be able to stratify breast cancer patients, firstly in order to restrict chemotherapeutic drug administration only to those who will benefit from it, and secondly to allow informed decisions regarding the choice of drug for each patient. As stated by Joe Nevins (Duke University Medical Center, Durham, USA), by finding and focusing on the specific subset of patients that will probably benefit from an individual therapy, a potential failed drug can become a blockbuster drug. Nevins presented gene expression profiles resulting from exposure to the major cancer drugs; from these, his group could generate signatures that represent a likely response or lack of response to individual therapies. They could use this approach to predict patients who would benefit from particular drug regimes. They are currently using these genomic screening tools in trials. Similarly, Bernards discussed gene expression signatures that predict outcome in women with breast cancer. He also showed data from a 159 gene signature of activated phosphatidylinositol 3-kinase, which was used to predict outcome in colon cancers. Bernards also showed data from a short hairpin RNA (shRNA) library screen to find genes

involved in resistance to trastuzumab (known as Herceptin) in a BT474 breast cancer cell line model. By simultaneously screening 24,000 human shRNAs against 8,000 genes, his group could identify genes required for trastuzumab effectiveness. They identified and validated the phosphatase pTEN as an essential component in the trastuzumab response.

Clearly, the single gene or single protein approach is rapidly becoming redundant. The use of screens allows researchers to simultaneously assess all genes, identify thousands of regulatory sites, test a multitude of compounds and combine these different screens in multifactorial ways. By distilling this information we can progress more rapidly towards personalized treatments.

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